

THE INFLUENCE OF BODY WEIGHT ON GAS EXCHANGE IN
THE AIR-BREATHING FISH, Clarias batrachus

By

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TABLE OF CONTENTS

	Page
Acknowledgements.	ii
List of Tables.	iv
List of Figures	v
Abstract.	vii
Introduction.	1
Materials and Methods	7
Results	10
Discussion.	37
Summary	58
Bibliography.	60
Biographical Sketch	63

LIST OF TABLES

	Page
1. The Metabolic Rate of Various Air-Breathing Fishes (and <u>Ictalurus nebulosus</u>) Under Varying Experimental Conditions.	39
2. Oxygen Capacities of the Blood of Representative Air-Breathing Fishes	43

LIST OF FIGURES

	Page
1. Metabolic rate of forcibly submerged <u>C. batrachus</u> as a function of body weight.	12
2. Metabolic rate of air exposed <u>C. batrachus</u> as a function of body weight	14
3. Metabolic rate of <u>C. batrachus</u> , in water with access to air, as a function of body weight.	16
4. Metabolic rates of <u>C. batrachus</u> (forcibly submerged, air exposed, water with access to air) and poikilotherms (from Hemmingsen, 1960) as a function of body weight. . . .	18
5. Metabolic rate of <u>C. batrachus</u> weighing 42 g as a function of oxygen tension.	21
6. Metabolic rate of <u>C. batrachus</u> weighing 77 and 83 g as a function of oxygen tension	23
7. Metabolic rate of <u>C. batrachus</u> weighing 172, 195 and 206 g as a function of oxygen tension	25
8. Relationship between oxygen uptake from air and oxygen tension of the water in <u>C. batrachus</u> weighing 30-71 g	28
9. Relationship between oxygen uptake from air and oxygen tension of the water in <u>C. batrachus</u> weighing 141-210 g	30
10. Diurnal and nocturnal surfacing frequency as a function of body weight	33
11. Average number of surfacings per hour as a function of time of day in air-saturated water and nitrogen-saturated water	35
12. Gill surface area as a function of body weight for various aquatic respiring teleosts (from Muir, 1969) and <u>C. batrachus</u> (from Saxena, 1966).	41

13.	Critical oxygen tension of <u>C. batrachus</u> and two salamanders, <u>Siren</u> (from Ultsch, 1972) and <u>Desmognathus</u> (from Beckenbach, 1969), as a function of body weight	47
14.	Gill surface area of <u>C. batrachus</u> as a function of body weight (from Saxena, 1966).	51
15.	Buffering capacity of blood of selected vertebrates at 25°C (from Howell, 1970, and Rahn, 1967)	55

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Aerial respiration and its capacity for terrestrial locomotion have enabled Clarias batrachus, a clariid catfish from southern Asia, to establish a large breeding population in southeastern Florida. Clarias batrachus is a facultative air breather, and at aquatic oxygen tensions exceeding 40 mmHg, an oxygen regulator.

The rate of metabolism of Clarias varies as a function of weight, and access to air. Aerobic metabolism is primarily utilized, even during forcible submergence when the metabolic rate declines. Clarias, a terrestrially active fish under certain environmental conditions, shows no metabolic reduction upon emersion.

The aquatic oxygen tension below which Clarias becomes an oxygen conformer is directly proportional to body weight, and about 1/3 the value for bimodally breathing salamanders; the disparity is presumably the result of the greater surface area for gaseous exchange in Clarias.

The number of air breaths per unit time doubles at night and is inversely proportional to body weight.

The sensitivity of its naked skin to desiccation and the low bicarbonate buffering capacity of its blood limit the terrestrial activity of Clarias to rainy nights when a cutaneous film of water can be maintained.

INTRODUCTION

The transition from aquatic to aerial breathing was one of the most important events in vertebrate evolution. Air breathing has evolved in numerous taxonomically unrelated groups of teleosts, and has produced a remarkable degree of convergence in these fishes. Bimodal breathers run the respiratory gamut from facultative air breathers that can survive indefinitely on the oxygen dissolved in water (except in times of severe oxygen stress), to obligate air breathers (such as Electrophorus, Lepidosiren, and Protopterus) that will drown when denied access to air; hence this is an excellent group in which to study the physiological modifications imposed by bimodal respiration. One such bimodally breathing teleost, Clarias batrachus, the "walking catfish," is the subject of this study. Air breathing has enabled this exotic introduction from southern Asia to become one of the dominant fishes in southeastern Florida; hence this species is an interesting form in which to study some of the aspects of bimodal respiration that have contributed to its success.

The two respiratory media, water and air, differ greatly in their physical properties, imposing very different morphological and physiological adaptations on the structure and function of respiratory surfaces. Water is a medium that often offers marginal conditions for gas exchange. It is frequently oxygen deficient. At 25°C, oxygen attains only 1/35 the concentration in water that

it does in air, but carbon dioxide is 27 times more soluble in water than oxygen, and may reach concentrations greatly in excess of aerial values. Consequently water breathers must ventilate much larger volumes of a denser medium than air breathers for a similar oxygen uptake. Air, in contrast, with its high partial pressure of oxygen offers a favorable diffusion gradient but requires significant structural support for respiratory surfaces. Furthermore, an atmospheric humidity below saturation will cause evaporation from the epithelial surfaces that must remain moist for gas exchange. Therefore a premium is placed upon structures protected within the body for aerial gas exchange. Obligate air breathers also must have an increased efficiency of the internal buffering system because of the elevated internal carbon dioxide tensions.

Since the differences in gaseous composition between air and water increase with temperature, it is not surprising that the majority of extant air-breathing fishes live in tropical freshwaters. These relatively warm waters usually have low oxygen and high carbon dioxide concentrations, due to the lack of turnover (because there is little wind disturbance and night temperatures are not cool enough), the reduction of aquatic photosynthetic activity because of shading, and the high B.O.D. of such waters.

Only a few temperate fresh-water species exhibit air breathing (Lepisosteus, Amia, and Umbra). Johansen (1970) demonstrated that air breathing in Amia calva increases at higher temperatures. The few marine air-breathing fish [some members of the families Gobiidae (including the mud skippers) and Blennidae, the clingfish

Sicyases (Gordon et al., 1970), the eel, Anguilla (Berg and Steen, 1965), and tarpons (Elopidae)] are mostly estuarine species and probably often encounter deoxygenated water in areas of organic decomposition such as mud flats, mangroves and vegetation mats.

Air breathing has enabled many of these fish to not only inhabit water with unfavorable gas tensions, but even to leave the water and spend part of their time on land. Certain fish, such as Anguilla (Berg and Steen, 1965), Symbranchus (Johansen, 1966), Clarias batrachus (Smith, 1945; Das, 1927), Saccobranthus = Heteropneustes, Anabas and Ophiocephalus (Das, 1927), frequently make night sojourns from pond to pond through moist grass. Some of these fishes are thought to feed while making these excursions. The Chilean clingfish, Sicyases sanguinensis (Gordon et al., 1970) rests on rocks in the inter-tidal splash zone, while the more active mudskipper, Periophthalmus, actually leaves the water to escape predators and to feed (Johansen, 1970). Other fish, such as African species of Clarias may move from drying pools to permanent bodies of water. Desiccation is a problem faced by most of these fish when they leave the water, hence the extent of these land excursions is a function of weather conditions, time of day, and physical conditions of the area.

In India (Das, 1927) and in south Florida (Ogilvie, personal communication), great numbers of Clarias batrachus migrate in the rainy season, presumably for feeding purposes. Mookerjee and Mazumdar (1950) reported that Clarias batrachus rarely migrates

by a land route like Anabas, but frequently migrates in shallow running water. This account corroborates descriptions of its behavior in south Florida, and some of the conclusions reached in this study.

If they are unable to escape a drying pool, certain air-breathing fish burrow into the mud and become dormant. Estivation has been reported for Clarias (Das, 1927; Sterba, 1963), Saccobranthus (Das, 1927; Hughes and Singh, 1971), Ophiocephalus (Das, 1927), Amia (Neill, 1950), Symbranchus (Johansen, 1966), and the African and South American lungfishes, Protopterus and Lepidosiren. Clarias and Saccobranthus, another clariid, bury themselves in moist mud five to six inches below the surface (Das, 1927). But with the exception of Janssens' (1964) experiments on Protopterus, little is known of this state.

Most air-breathing fish are facultative air-breathers, employing aquatic breathing unless the water is deoxygenated. Willmer (1934) observed that high carbon dioxide concentrations also stimulate air breathing, even when the oxygen content is adequate for water breathing.

All air-breathing fish utilize aquatic gas exchange to some extent, because the air-breathing organs show a low gas exchange ratio. Since carbon dioxide is highly soluble in water, its elimination takes place primarily through the gills or the skin in naked forms. Rates of oxygen uptake vary, depending on the respiratory medium and dominant mode of respiration. Most air breathers show a reduction in total oxygen consumption when

breathing air alone, although this is not the case with obligate air breathers and forms that are active upon emersion, such as Periophthalmus.

The morphological and physiological modifications for bimodal breathing in part account for the ecological success of many of these species when introduced into new areas. Clarias batrachus, a clariid catfish that is widely distributed from Ceylon through eastern India to the Malay Archipelago (Sterba, 1963), was introduced into the United States for the aquarium trade and escaped from a "fish farm" near Fort Lauderdale, Florida, probably in 1966 or 1967. Because of its capacity for air breathing and mobility, C. batrachus has spread via drainage ditches and canals throughout much of southeastern Florida.

This study attempts to examine some of the aspects of respiratory physiology of C. batrachus that have contributed to the success of this species in south Florida. The breathing response of the animals as a function of forcible submergence, oxygen tension, time of day, and body weight was examined. Measurements were made of the rate of oxygen uptake ($\dot{V}O_2$) under various experimental conditions (forcible submergence, air exposure, and water with access to air) and related to the ecology and behavior of this species. An attempt was made to determine if C. batrachus utilized anaerobic glycolysis when the oxygen consumption was reduced during forcible submergence. Electrocardiograms were taken to compare the cardiac response of C. batrachus to those of other air breathing fish, and an attempt was made to induce Clarias to estivate.

While this study was in progress, Singh and Hughes (1971) published results of a similar study of C. batrachus that were imported from India. Although there was some overlap in the scope of the two studies, the findings of Singh and Hughes provide valuable comparative data that augment this study.

MATERIALS AND METHODS

An electro-shocking apparatus and dip nets were used to collect specimens of C. batrachus in the vicinity of Fort Lauderdale, Florida. Fish were maintained in aquaria at $25 \pm 2^{\circ}\text{C}$ on a twelve light/twelve dark photoperiod.

All metabolic rates were determined at $25 \pm 1^{\circ}\text{C}$. Experiments were conducted during the day to obtain values for resting metabolism, as Clarias is nocturnal. In a few cases, as during partitioning of aquatic and aerial phases of respiratory exchange, experiments continued into the evening. All values for oxygen consumption were converted to $\text{ccO}_2/\text{kg-hr}$ at STP.

Oxygen uptake of submerged animals was determined through the use of a chamber (Erlenmeyer flasks or sealed battery jars) from which samples of water were withdrawn through polyethylene tubing. A Radiometer PHM 71 Acid-Base Analyzer was used to measure the PO_2 of the water sample. Animals were acclimated to the chamber at least eight hours prior to a determination.

Aerial respiration was determined by manometry. The apparatus consisted of two equal volume battery jars, serving as the metabolic and compensation chambers, respectively. A container of Soda Sorb was placed in the fish chamber to absorb the expired carbon dioxide. Oxygen used in the metabolic chamber was replaced by means of a syringe. As long periods of air exposure produced

skin necrosis, animals were introduced into the chamber for only an hour before metabolic determinations were made. To determine cutaneous oxygen uptake in air, a latex condom was placed over the head of the fish, preventing gas exchange through the air breathing organ and gills.

Rates of oxygen uptake in water where the fish had free access to air were measured in a two section chamber. A small opening separated the aquatic from the aerial phase. A 1/4 inch mesh funnel guided the surfacing fish to this opening. Water samples were drawn from the aquatic chamber for PO_2 determinations, and aerial respiration was determined manometrically. In a control using water equilibrated with nitrogen there was no significant amount of diffusion of oxygen into the aquatic phase.

The frequency of aerial breathing as a function of body weight and time of day and/or amount of incident radiation was determined with a Hunter Model 3355 photorelay photo cell coupled to an Esterline Angus event recorder. Air-equilibrated water was circulated to the recording chamber to keep the PO_2 and PCO_2 levels constant.

Experiments were conducted to determine whether anaerobic pathways made a significant contribution to the total energy expenditure of forcibly submerged animals. Studies of fish intermediary metabolism (Hochachka, 1969) indicate that the Embden-Meyerhof pathway is the principal pathway of glucose catabolism during aerobic metabolism, with the hexose monophosphate shunt providing a minor contribution, but during anoxia the EMP is

operative exclusively. In order to prevent nearly all anaerobic energy production without greatly interfering with aerobic processes, fish were injected with iodoacetic acid, which irreversibly inhibits phosphoglyceraldehyde dehydrogenase and thus blocks the Embden-Meyerhof pathway prior to any energy release. In one experiment, fish were injected intraperitoneally with a dosage of 15 mg per kg of IAA after Belkin (1961). Another group was poisoned with a dosage of 20 mg per kg, after Rose and Drotman (1967). Half the poisoned fish (and unaltered controls) were then forcibly submerged in air-equilibrated water and half were allowed access to air to determine whether such poisoning was detrimental to submerged fish.

Electrocardiograms were recorded on a Grass Model 79 polygraph. Two leads were inserted into the fish's body on either side of the heart and tied dorsally. Fish were anesthetized with MS-222 to facilitate electrode implantation, and allowed to recover for several hours before recordings were made.

Estivation chambers were constructed from a nylon mesh bag placed inside a hardware cloth frame that was placed inside a battery jar or refrigerator liner. Mud was placed in the bag, and the container filled with water. The water was drained off at the rate of an inch every other day. Several different mud substrates were used, and in some cases a bright light was left on the fish during the day.

RESULTS

Clarias batrachus weighing 69 to 178 g survived at least two weeks when forcibly submerged in air-saturated water. This species can also survive out of water for extended periods, provided the humidity is high and the skin is kept moist. A 54 g individual survived 3 days and a 124 g individual survived 4 days at 21°C when kept in plastic boxes with wet cheesecloth. Individuals exposed to room air succumbed in about six to eight hours. The skin of these individuals rapidly dried during this period.

The rates of oxygen consumption varied greatly as a function of body weight and access to air. The metabolic rate of forcibly submerged Clarias can be described by the relationship $\text{Log } M = 0.78 \text{ Log } W - 0.82$ ($r = 0.96$; see Fig. 1). Their metabolic rates in air are described by the relationship $\text{Log } M = 0.72 \text{ Log } W - 0.54$ ($r = 0.87$; see Fig. 2). When the fish were placed in water and given access to air their rates of metabolism are described by the relationship $\text{Log } M = 0.63 \text{ Log } W - 0.36$ ($r = 0.93$; see Fig. 3). In Fig. 4 the three curves (forcibly submerged, aerial and water with access to air) are compared. These curves are essentially identical in their power of weight, but the curve for forcibly submerged animals is lower than for individuals that have air available for gas exchange. The relation of standard energy metabolism to weight for poikilotherms, corrected to 25°C ($\text{Log } M = 0.75 \text{ Log } W - 0.69$, from Hemmingsen, 1960) is also shown in Fig. 4.

Figure 1. Metabolic rate of forcibly submerged C. batrachus
as a function of body weight.

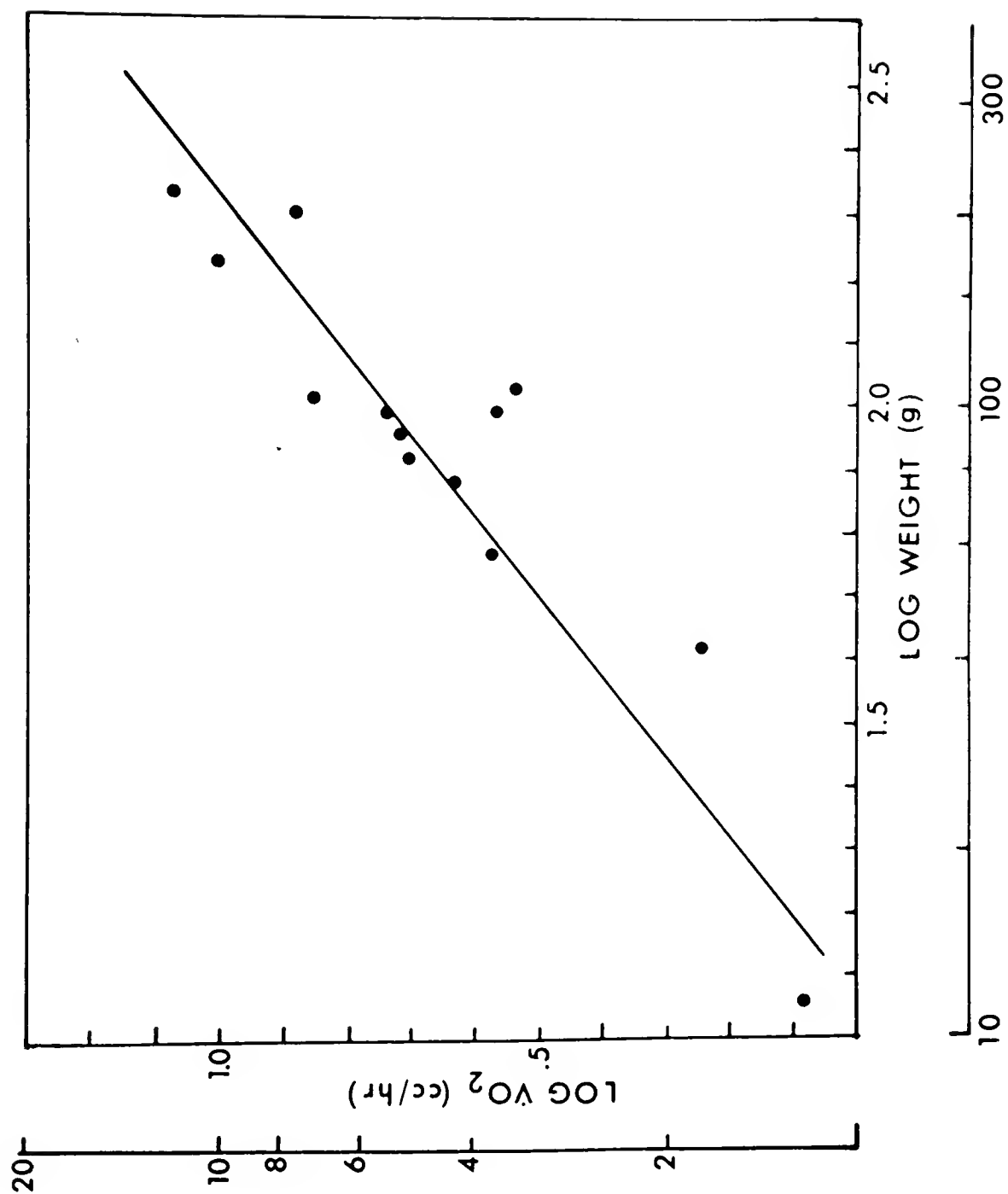


Figure 2. Metabolic rate of air exposed C. batrachus
as a function of body weight.

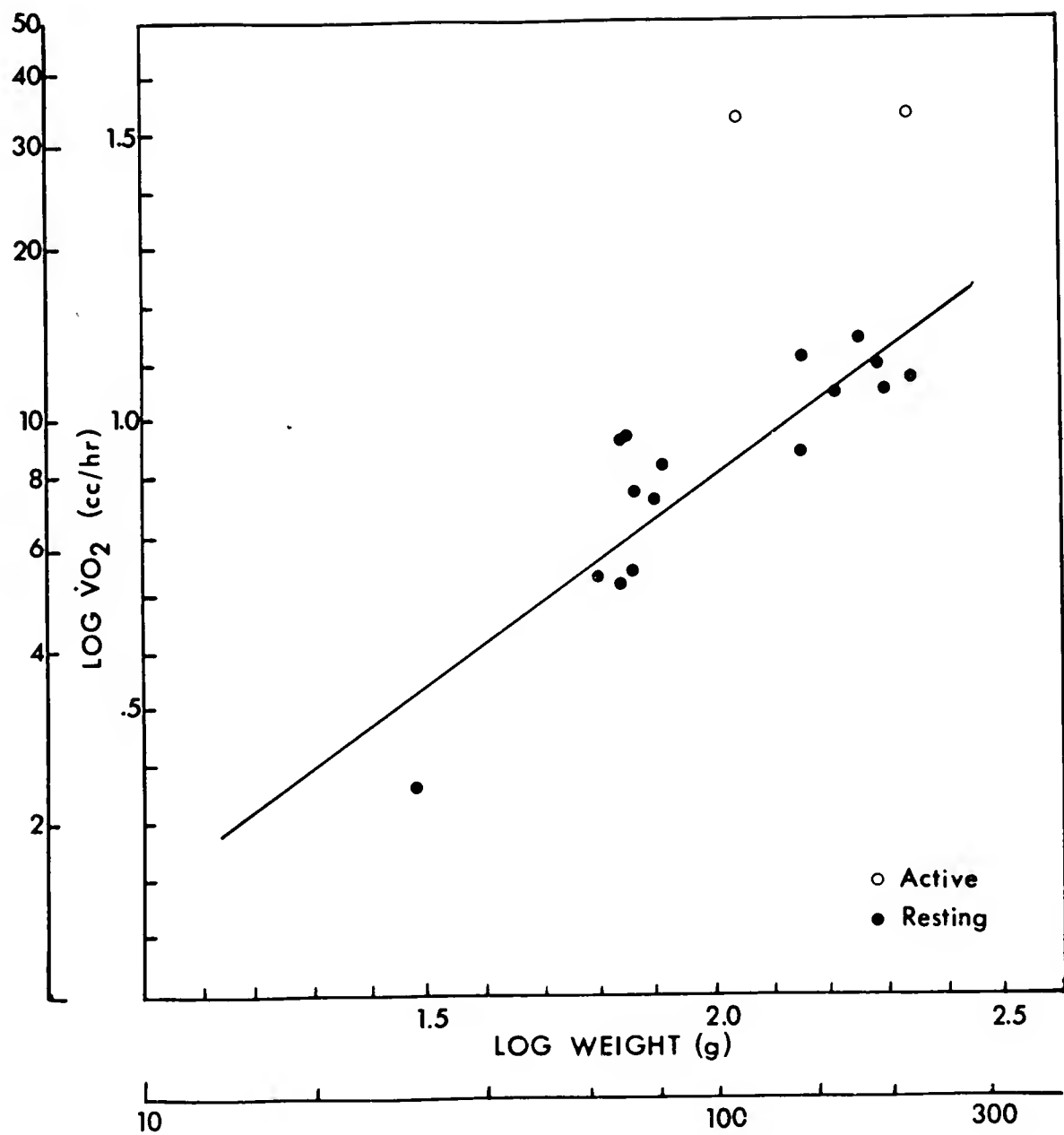


Figure 3. Metabolic rate of C. batrachus, in water with access to air, as a function of body weight.

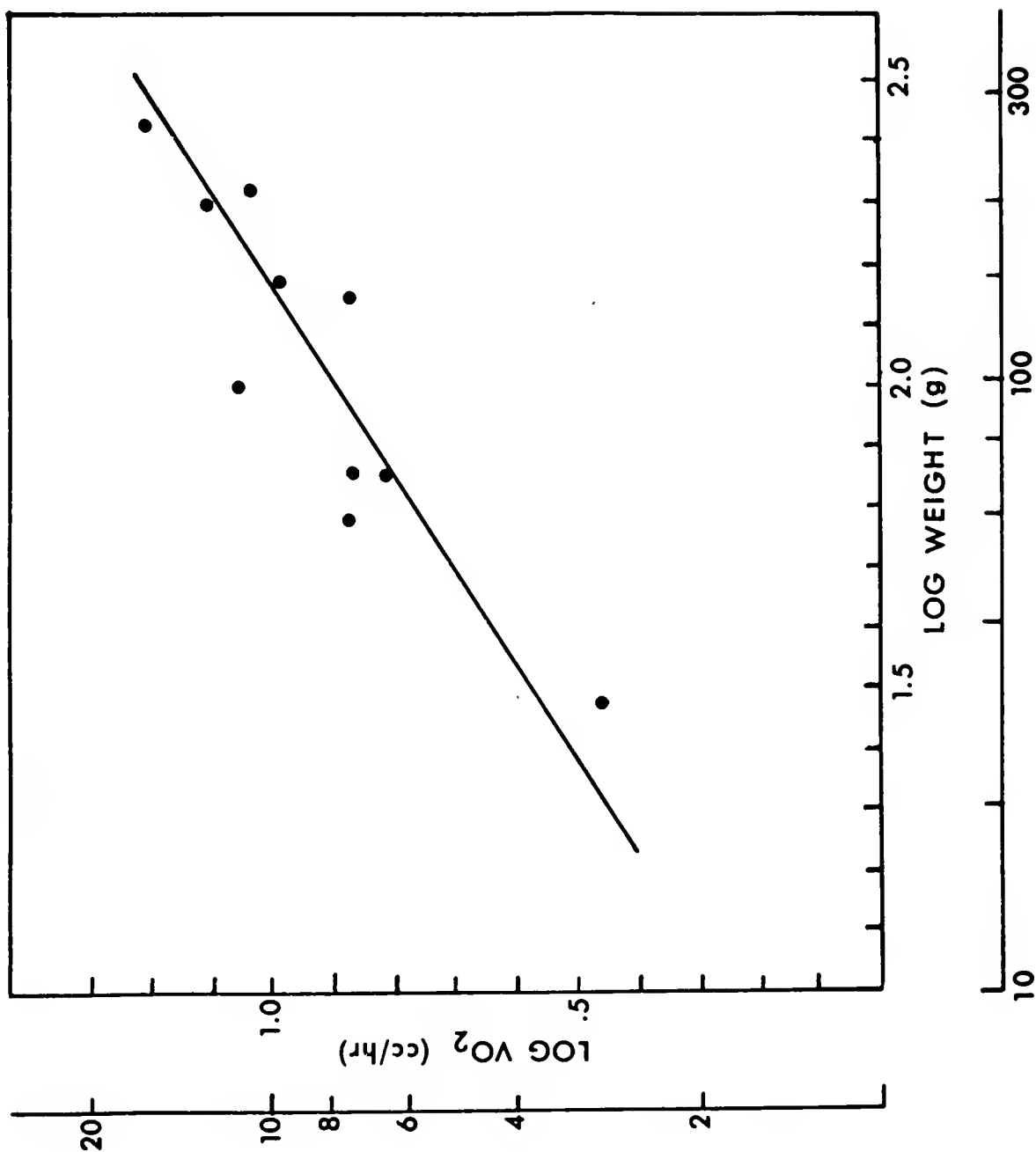
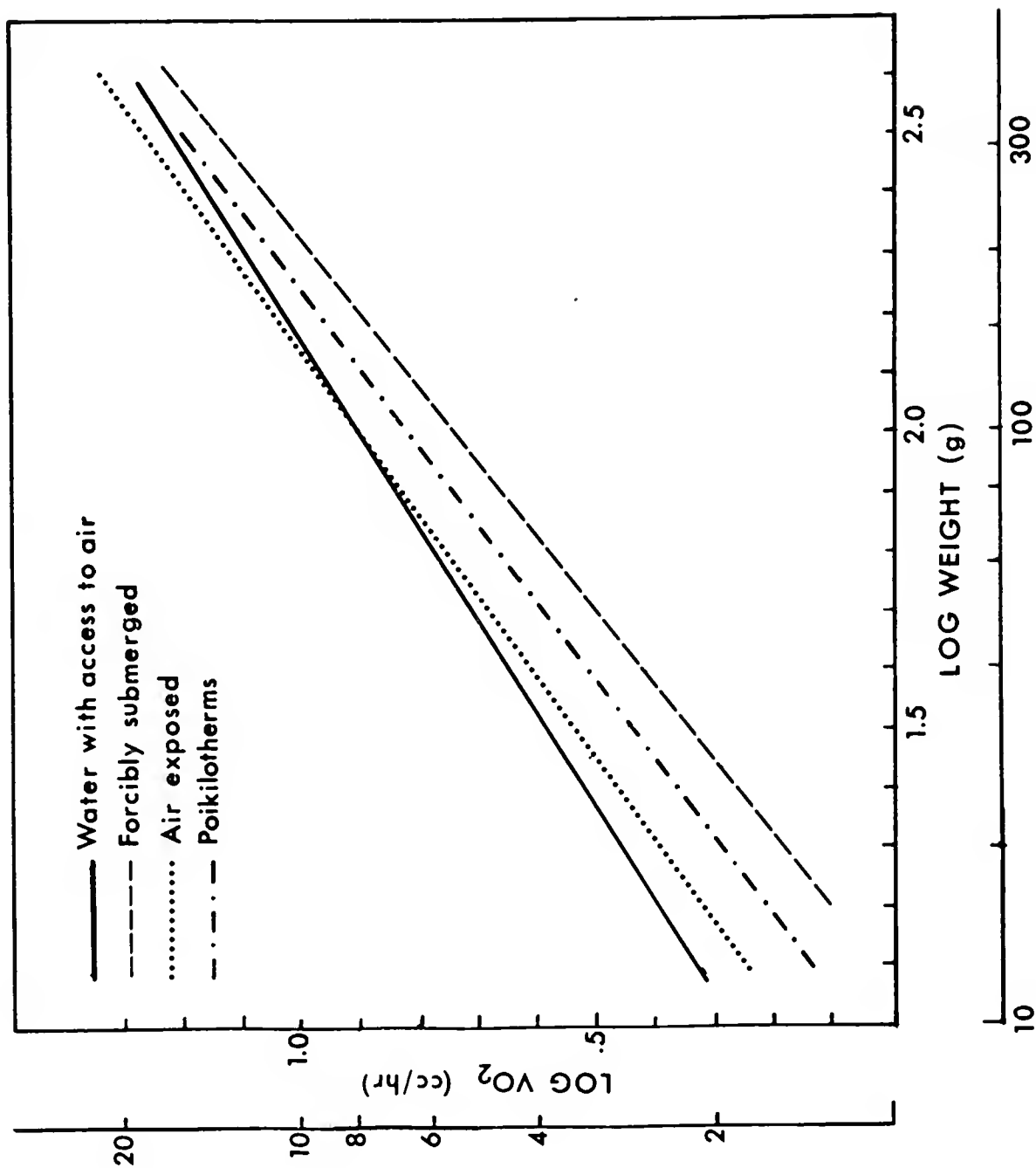


Figure 4. Metabolic rates of C. batrachus (forcibly submerged, air exposed, water with access to air) and poikilotherms (from Hemmingsen, 1960) as a function of body weight.



Active metabolism of a few air exposed individuals was obtained; values for a 220 g and a 113 g individual were 148.2 ccO₂/kg-hr and 290.2 ccO₂/kg-hr respectively, as compared to resting rates of 52.5 ccO₂/kg-hr and 78.5 ccO₂/kg-hr, respectively (Fig. 2). Activity then may raise metabolism at least by a factor of three. Cutaneous respiration accounted for up to 50% of the total oxygen uptake of two large (200 and 220 g) air exposed fish.

The effects of gradual hypoxia on metabolism of submerged Clarias weighing 42 to 206 g is shown graphically in Figs. 5, 6, and 7. The metabolic rate appears to be independent of oxygen concentration over the range of PO₂'s from saturation to about 40 mmHg. At oxygen tensions less than 40 mmHg these fish appear to be oxygen conformers.

As submerged fish exhibited the lowest rates of oxygen uptake, the question arose whether these fish might depend upon anaerobiosis. Four fish were poisoned with a 15 mg Iodacetate per kg body weight; two fish were forcibly submerged in air-saturated water, and the other two were placed in air-saturated water with access to air. One unpoisoned fish was placed with each group. Weights of the individuals used are given below:

<u>Submerged</u>	<u>Access to Air</u>
113 g poisoned	156 g poisoned
153 g poisoned	190 g poisoned
178 g normal	163 g normal

The 153 g individual died after two days, all others survived for 14 days, when the experiment was terminated. This experiment was

Figure 5. Metabolic rate of C. batrachus weighing 42 g as a function of oxygen tension.

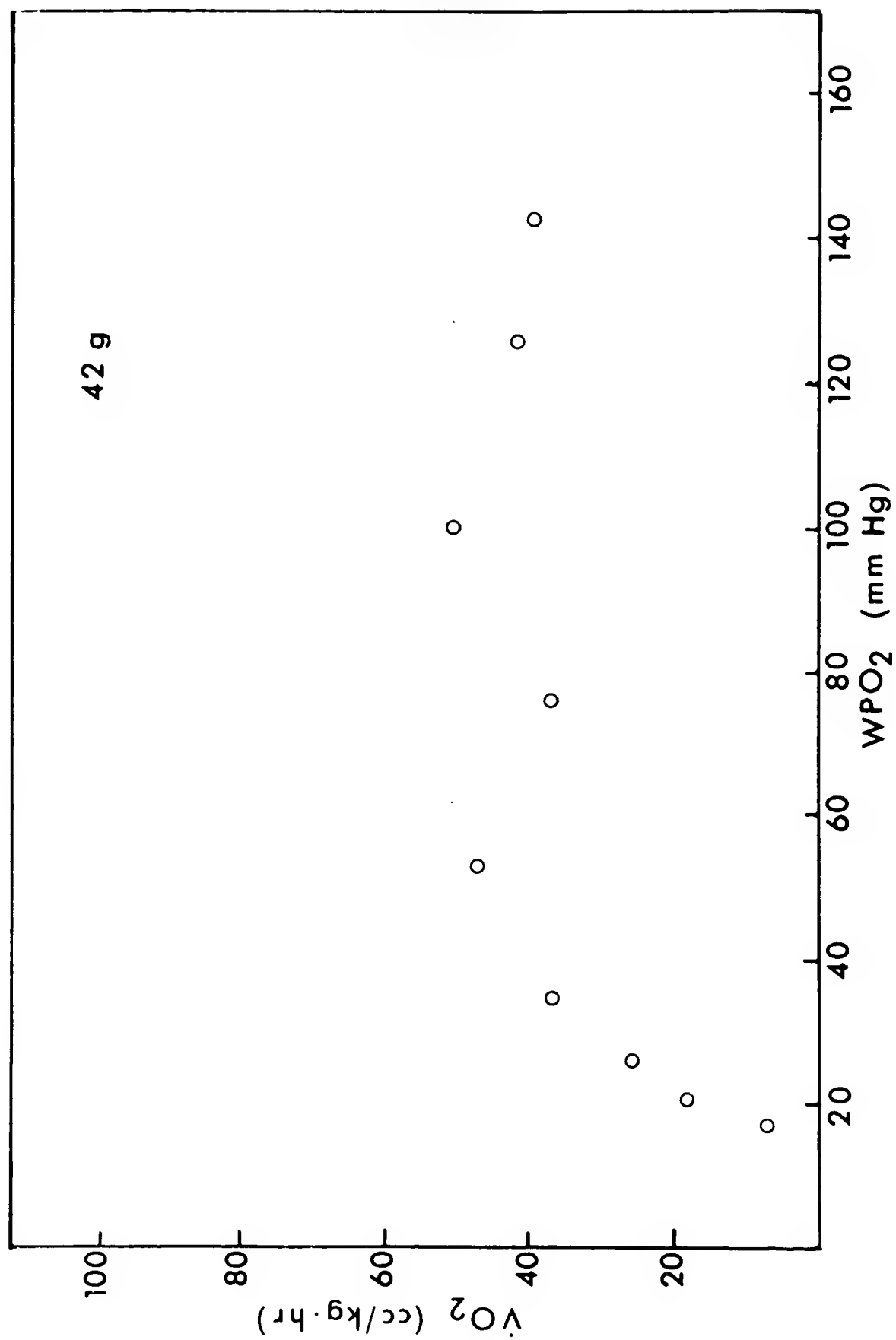


Figure 6. Metabolic rate of C. batrachus weighing 77 and 83 g
as a function of oxygen tension.

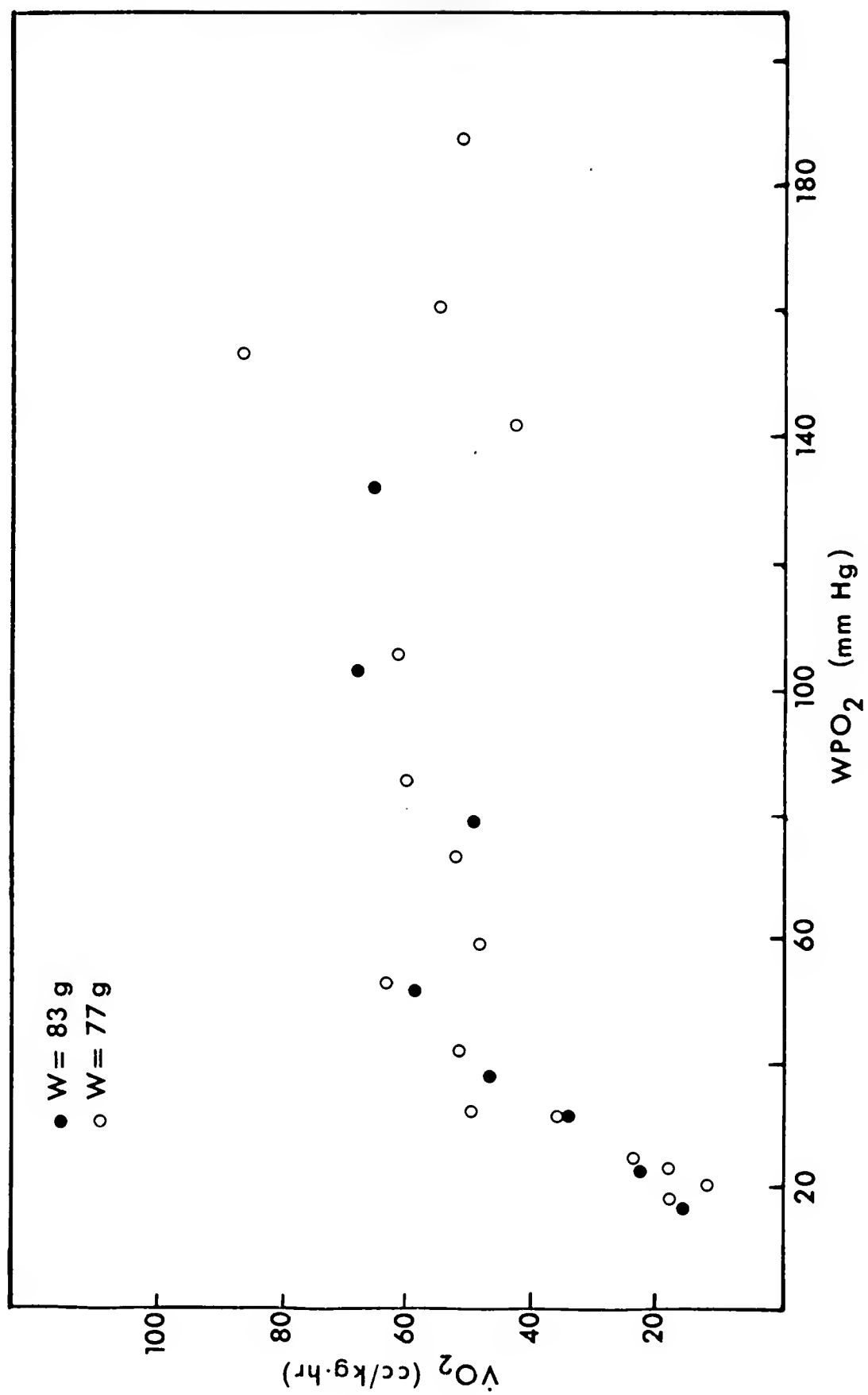
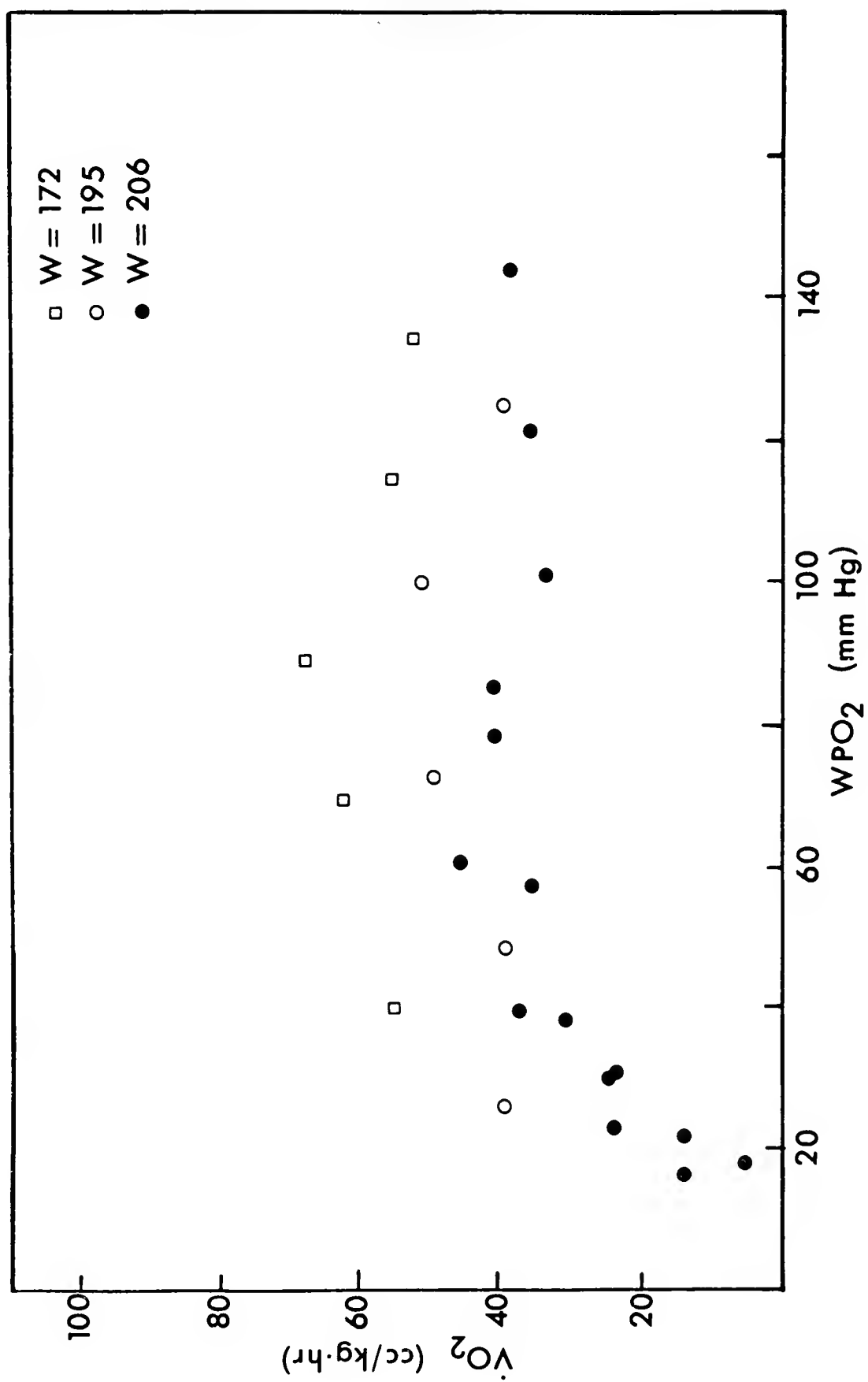


Figure 7. Metabolic rate of C. batrachus weighing 172, 195 and 206 g as a function of oxygen tension.



repeated using seven fish and a dosage of 20 mg/kg. The weights of these fish are given below:

<u>Submerged</u>	<u>Access to Air</u>
77 g poisoned	99 g poisoned
132 g poisoned	181 g poisoned
164 g poisoned	139 g poisoned
180 g poisoned	140 g normal
69 g normal	
224 g normal	

The 181 g and 139 g individuals, which had access to air went into tetany when placed in the tank and died in a few hours. The 77 g individual died during the night. All others survived for two days and the experiment was terminated. The fact that most of the submerged animals that were poisoned survived would tend to preclude a significant dependence on anaerobiosis when this species is denied access to air in normoxic water.

The percentage of oxygen uptake from air when the fish were in water of various O_2 concentrations with access to air is shown in Figs. 8 and 9. Aquatic breathing accounted for 80% to 90% of the total oxygen uptake at higher oxygen tensions. At lower oxygen tensions aerial respiration increases. Smaller fish appear to increase the amount of aerial respiration at higher oxygen tensions than larger fish, as the decrease in percentage of aquatic respiration for smaller fish occurs when oxygen tensions are about 60 mmHg (Fig. 8) as opposed to 40 mmHg for larger individuals (Fig. 9).

The total oxygen uptake is reduced when Clarias is kept in deoxygenated water ($PO_2 = 6$ mmHg) with free access to air. The mean $\dot{V}O_2$ of a 141 g individual was 39.1 cc O_2 /kg-hr compared to a level of

Figure 8. Relationship between oxygen uptake from air and oxygen tension of the water in C. batrachus weighing 30-71 g.

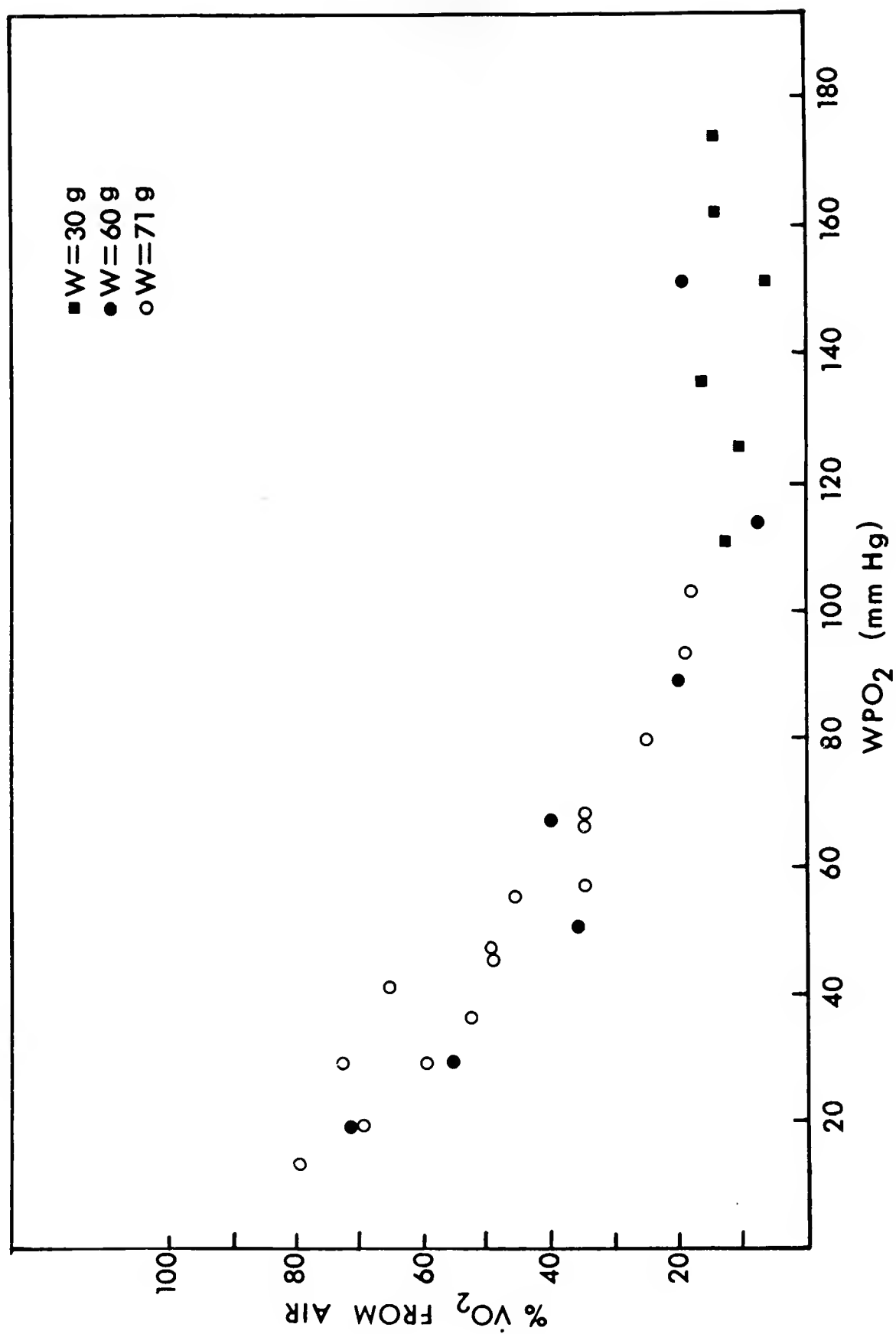
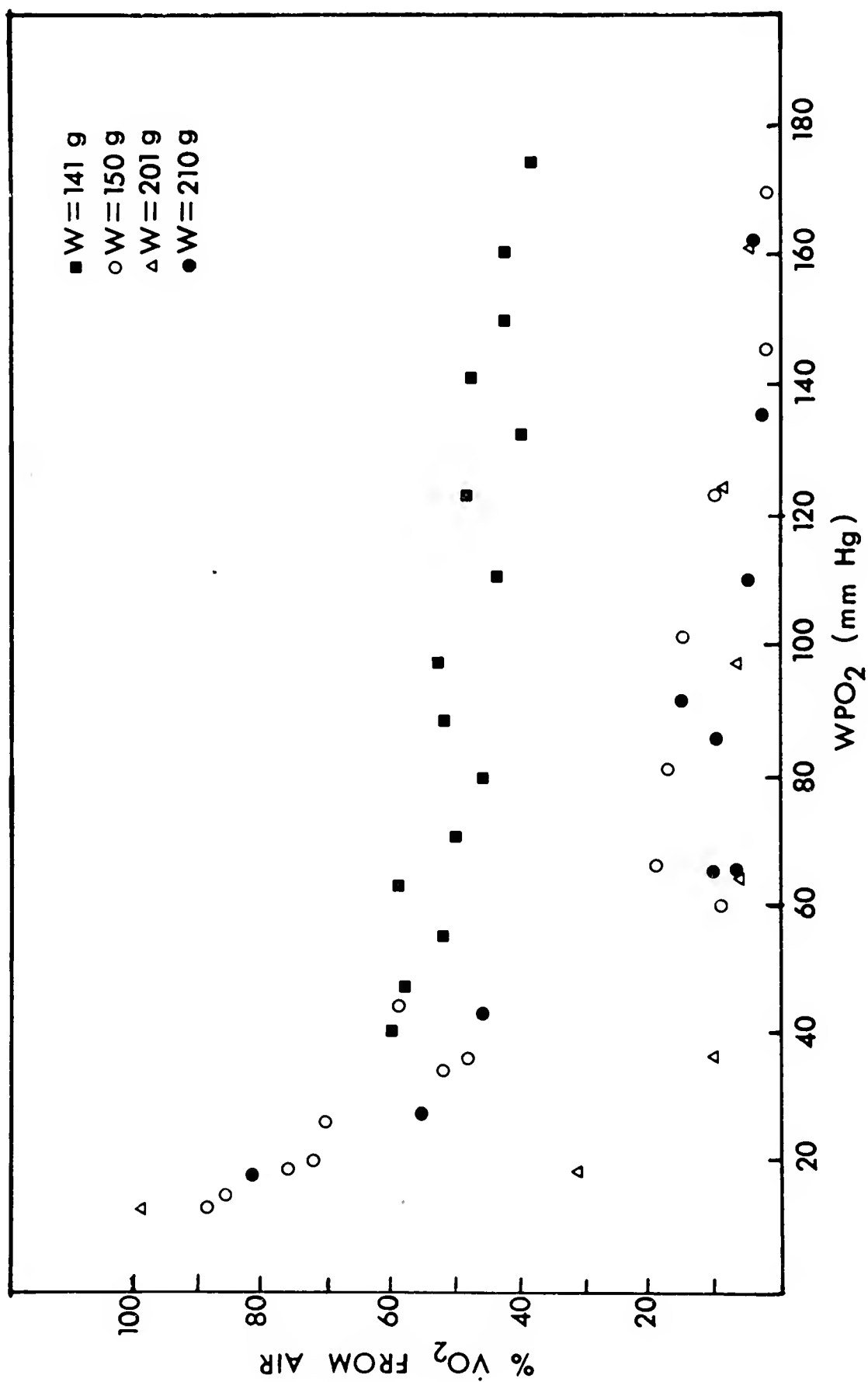


Figure 9. Relationship between oxygen uptake from air and oxygen tension of the water in C. batrachus weighing 141-210 g.



53.5 ccO₂/kg-hr in normoxic water. A 71 g individual had a rate equal to 51.4 ccO₂/kg-hr, compared to a normal level of 94.2 ccO₂/kg-hr. Clarias proved capable of raising the $\dot{V}O_2$ under these conditions; the 71 g fish had an aerial $\dot{V}O_2$ of 93.8 ccO₂/kg-hr when active in the dark at a water PO₂ that averaged 28 mmHg. This increase was due to increased aerial gas exchange only. Clarias can survive for weeks living in oxygen-deficient water if it has access to air.

The number of air breaths taken per hour is inversely proportional to the weight of the fish raised to a power less than one and is influenced by the time of day (and amount of incident radiation) (Fig. 10). The mean number of daytime surfacings for a 30 g individual was 19, while that of a 244 g individual was 6. The surfacing frequency increased by 60% to 100% at night. Lowest values were between 1100 and 1400 hours EST. On cloudy or rainy days, surfacings were similar to night values. Any disturbance (turning on the light at night, standing near the apparatus) caused surfacing frequency to decline. An 80 g fish placed in nitrogen-saturated water surfaced about the same number of times during the day as it did in air-saturated water, but at night the number of surfacings doubled (see Fig. 11).

Several attempts were made to induce estivation in Clarias. Many constructed and used burrows that extended three or four inches into the mud. Several individuals survived for a period of three to four days in the estivation chambers, but invariably

Figure 10. Diurnal and nocturnal surfacing frequency as a function of body weight.

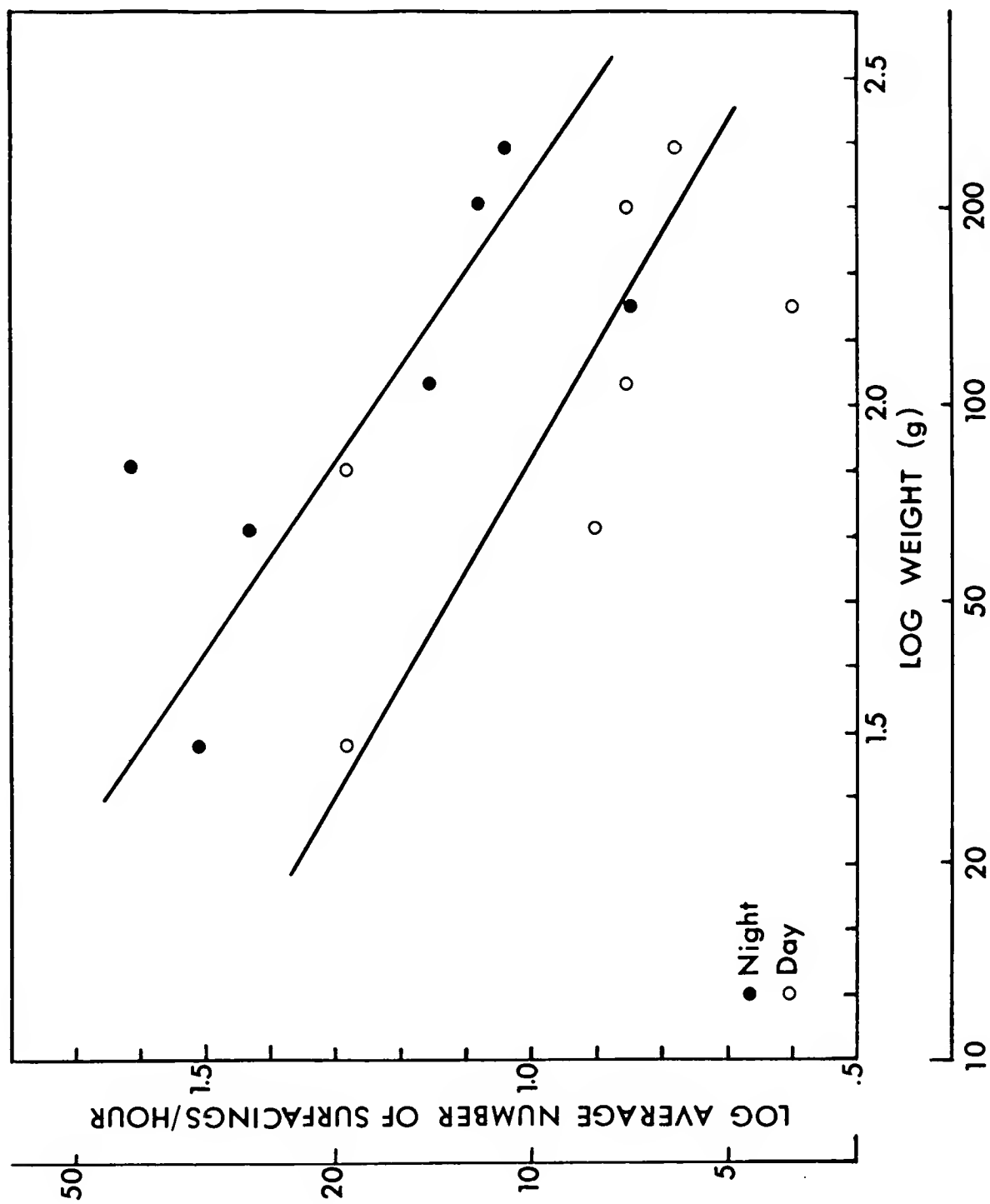
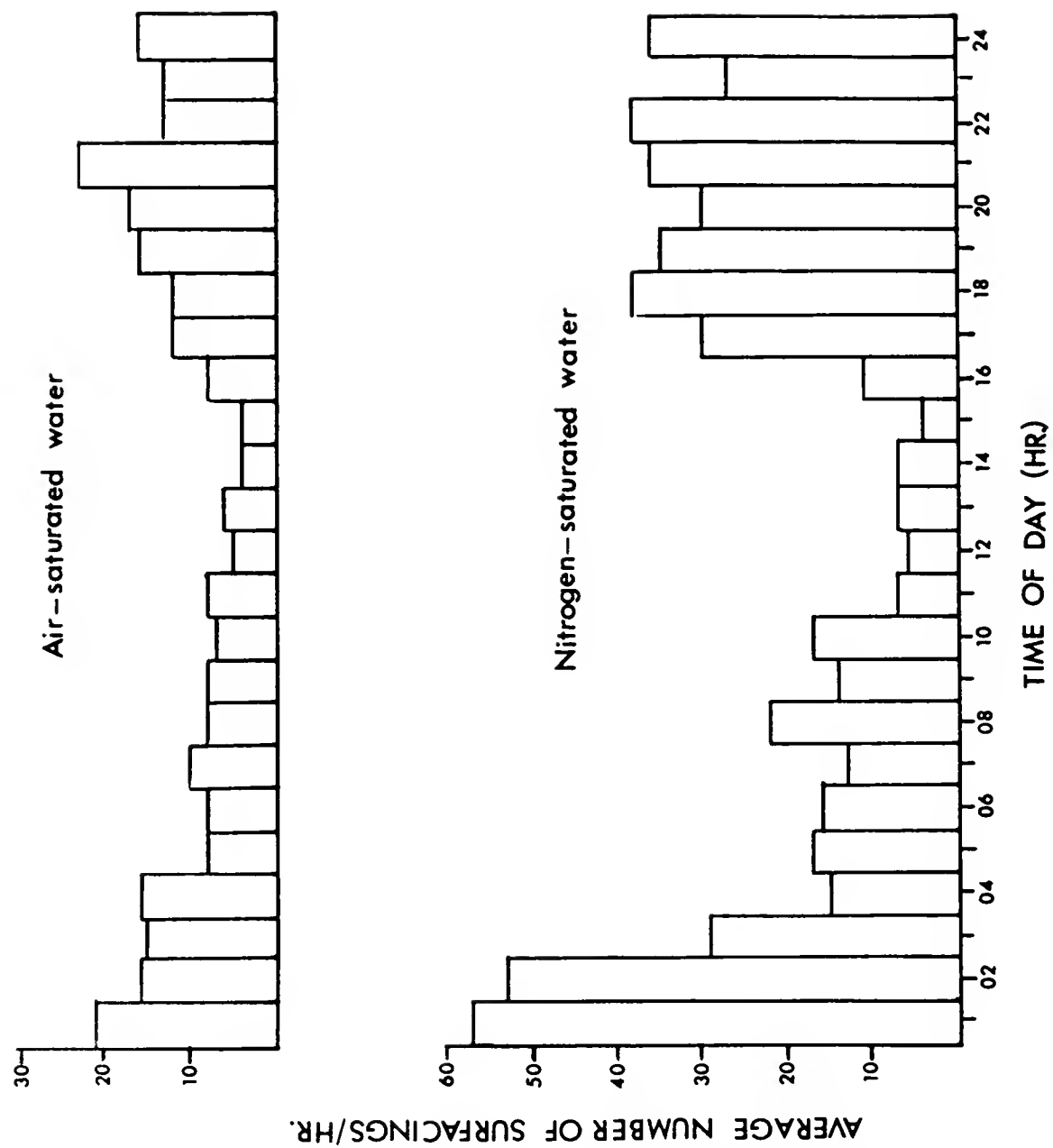


Figure 11. Average number of surfacings per hour as a function of time of day in air-saturated water and nitrogen-saturated water.



with the fall in the water level, they emerged, desiccated and died.

Electrocardiographic measurements of heartbeats demonstrated some physiological changes upon emersion. Heartbeat frequencies of a water-breathing fish were around 30 beats/minute and rose to 39 beats/minute following an air breath. When the water was drained from the tank, bradycardia (15 beats/minute) ensued for about three minutes, until the fish moved and gulped air. This was followed by a tachycardia of 78 beats/minute.

DISCUSSION

Clarias batrachus appears to be a facultative air breather, as forcibly submerged animals survived at least two weeks. Saxena (1960) also showed that small C. batrachus and S. fossilis could survive forcible submergence for at least two weeks. Clarias batrachus has been described by Singh and Hughes (1971) and Das (1927) as an obligate air breather. Singh and Hughes (1971) provided no data to support this supposition, and Das' fish succumbed after two hours when a copper screen was used to prevent access to the surface. It is possible that copper poisoning, rather than air deprivation, caused the demise of the fish used in Das' experiments.

The energy requirements of a fish, which are reflected by the oxygen consumption, depend on several factors, including physical properties of the water (amount of oxygen, carbon dioxide, temperature) and the physiological, anatomical, and behavioral modifications of the fish (ventilation rate, area for gas exchange, activity). High ventilation rates, large areas for gas exchange, and an oxygen rich environment all contribute to high rates of oxygen extraction. Air-breathing fishes, which frequently inhabit waters of low oxygen tensions, and often show reduced areas for aquatic gas exchange, might be expected to have low metabolic rates. In Fig. 4, metabolic curves for C. batrachus (forcibly submerged, aerial and water with access to air) are compared to the relation of standard energy

metabolism to weight for poikilotherms, corrected to 25°C (from Hemmingsen, 1960). The curve for forcibly submerged Clarias is slightly below the standard poikilotherm curve, while those for Clarias breathing air or water with access to air are slightly above.

The metabolic rates of C. batrachus and several other air-breathing fishes are shown in Table 1. The rates of oxygen consumption of air-breathing forms are usually labile, depending upon experimental conditions and are often lowered when the animals are submerged. In the case of Protopterus and Electrophorus, both obligate air breathers, the low metabolic rates in submerged animals are related to their small gill surface area. These fish will drown if denied access to air.

The lowering of $\dot{V}O_2$ in submerged Clarias is presumably not due to the utilization of any anaerobic pathways, as submerged IAA poisoned fish fared as well as those with access to air. Singh and Hughes (1971) state that the lowering of the total $\dot{V}O_2$ in submerged Clarias may be related to the reduced surface area of the gills and the thickness of the gill epithelium. Saxena (1966) found the gill area values of C. batrachus ($295 \text{ mm}^2/\text{gm}$) and of S. fossilis ($395 \text{ mm}^2/\text{gm}$) were small compared to values of open water forms (Rita rita = $1,000 \text{ mm}^2/\text{gm}$), but when values for Clarias are compared to data on additional species (Fig. 12, from Muir, 1969), its gill surface area surpasses those of many purely aquatic breathers—the carp, Cyprinus carpio; the white sucker, Catostomus commersoni; and the brown bullhead, Ictalurus nebulosus) Munshi and Singh (1968)

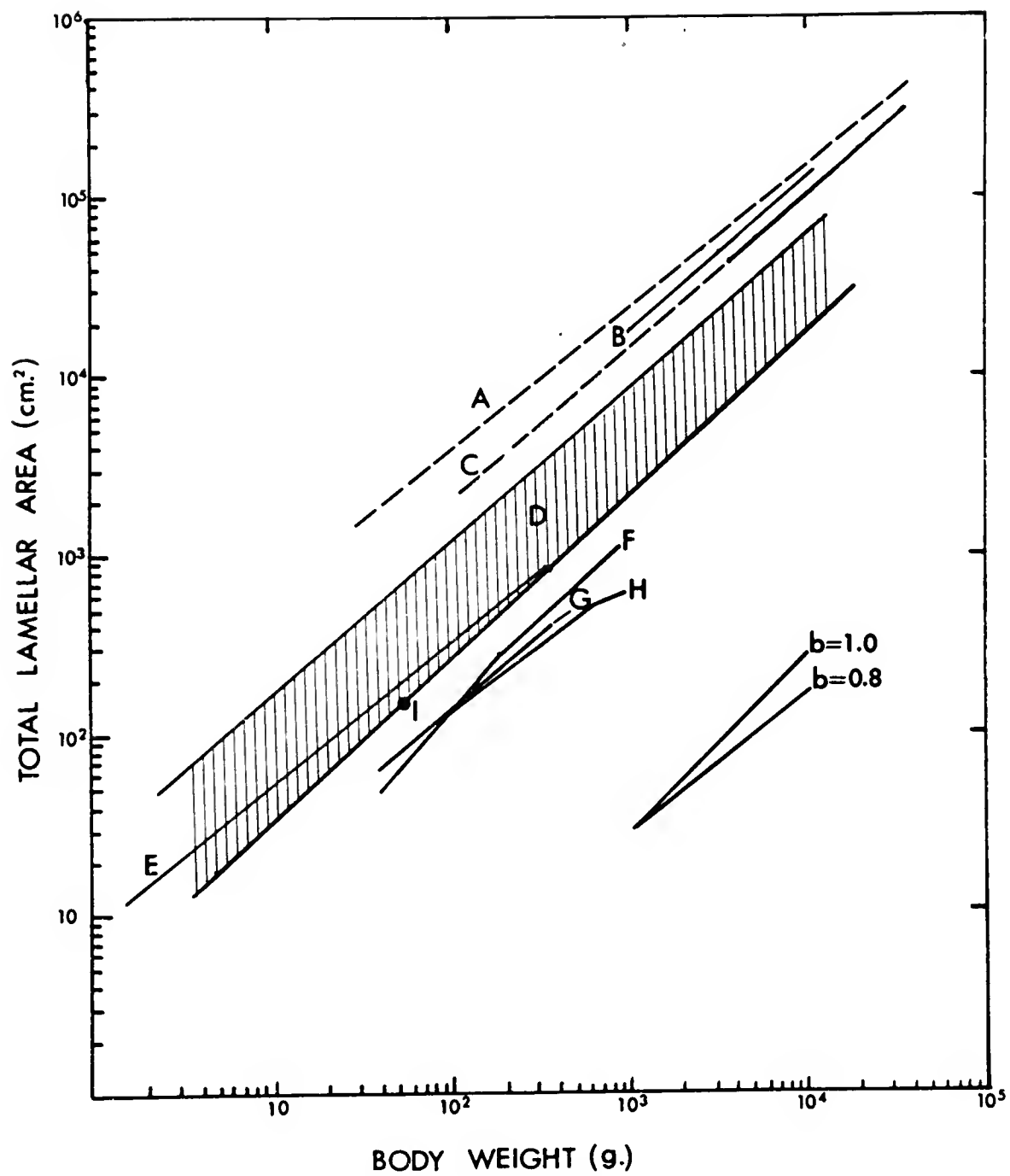
TABLE 1

The Metabolic Rate of Various Air-Breathing Fishes (and Ictalurus nebulosus) Under Varying Experimental Conditions

Species	Cont. Flow of Water	Water & Air	Air	Still Water	Import. of Air Breath.	Weight (gms.)	Temp.	Reference
<u>Anabus</u> <u>testudineus</u>	75.5	113.42	104 Active		Accessory	29-51	25°C	Hughes and Singh, 1970
<u>Anguilla</u> <u>vulgaris</u>	26.6		11.54		Accessory	475-580	15°C	Berg and Steen, 1965
<u>Clarias</u> <u>batrachus</u>	64.94	93.39	71.17	60.85	Accessory	87-157	25°C	Singh and Hughes, 1971
<u>Clarias</u> <u>batrachus</u>		79.4	79.4	54.9	Accessory	100	25°C	Present study
<u>Electrophorus</u> <u>electricus</u>		29.8	23.04	6.75	Obligate	2,760	26°C	Farber and Rahn, 1970
<u>Periophthalmus</u> <u>sobrinus</u>			94	84		1-5	25°C	Gordon et al., 1966
<u>Saccobranchus</u> <u>fossilis</u>	66.35	84.50	54.50	96.40	Accessory	48-62	25°C	Hughes and Singh, 1971
<u>Protopterus</u> <u>aethiopicus</u>	10	62.5			Obligate	100-600	24°C	McMahon, 1970
<u>Sicyus</u> <u>sanguinensis</u>			32	40	Accessory		15°C	Gordon et al., 1970
<u>Protopterus</u> <u>aethiopicus</u>		13.5	13.8		Obligate		20°C	Lenfant and Johansen, 1968
<u>Ictalurus</u> <u>nebulosus</u>	70				None	74.9	25°C	Marvin and Heath, 1968

Figure 12. Gill surface area as a function of body weight for various aquatic respiring teleosts (from Muir, 1969) and C. batrachus (from Saxena, 1966).

A	Mammals
B	Tuna
C	
D	Most teleosts
E	Bass
F	Toadfish
G	Sucker
H	Bullhead
I	<u>Clarias</u>



determined that the respiratory epithelium is very thick in C. batrachus ($8\ \mu$ - $15\ \mu$ as compared to $0.8\ \mu$ - $3.2\ \mu$ for water breathers). In addition, they estimated that the thickness of the basement membrane in C. batrachus precluded all but 52% of the surface area available for aquatic exchange. Even if Saxena did not account for the reduction in exposed secondary lamellar area, the proportional extent of the gill surface of Clarias still exceeds the values of carp, bullhead, and sucker.

The thickness of the epithelium in Clarias undoubtedly reduces the rate of diffusion of oxygen into the blood stream. But this is probably at least partially offset by the high oxygen capacity (18.0 vol.%, Singh and Hughes, 1971) of the blood. High oxygen capacity values probably enhance the ability of the blood to pick up more oxygen while passing through the gills. Johansen et al. (1968) hypothesized that the high oxygen capacity of Electrophorus blood was an adaptation to mixing of oxygenated and deoxygenated blood that results from the shunting of respiratory efferent blood to the systemic veins. Yet the blood of Symbranchus, Clarias, and Saccobranhus show the greatest oxygen capacity values (Table 2), and these species are among the few air-breathing fishes that have the ideal perfusion pattern (no mixing of arterial and venous blood) existing in purely aquatic-breathing fishes. All are facultative air breathers. Symbranchus has a highly reduced gill surface area ($40\ \text{mm}^2/\text{gm}$, Junqueira, Steen and Tinoco, 1967) and the gill lamellae are thickened so they do not collapse in air. Thus it may be that the high oxygen capacity enables these primarily

TABLE 2

Oxygen Capacities of the Blood of Representative
Air-Breathing Fishes

Species	Oxygen Capacity (vol. %)	Reference
<u>Clarias</u> <u>batrachus</u>	18.0	Singh and Hughes, 1971
<u>Saccobranhus</u> <u>fossilis</u>	17.5	Singh and Hughes, 1971
<u>Electrophorus</u> <u>electricus</u>	13.9	Johansen et al., 1968
<u>Lepidosiren</u> <u>paradoxa</u>	8.25	Johansen, 1970.
<u>Amia</u> <u>calva</u>	7.8	Johansen et al., 1970
<u>Protopterus</u> <u>aethipicus</u>	9.50	Lenfant and Johansen, 1968
<u>Symbranchus</u> <u>marmoratus</u>	17.30	Johansen, 1970

aquatic breathers to pick up more oxygen than would normally be possible through their reduced or thickened gill surfaces. A high oxygen capacity would also increase the interval between air breaths when oxygen tensions in water were low, by increasing the oxygen store of the body.

It appears that the low metabolic rates of submerged animals are at least partially due to a decrease in activity. Hughes and Singh (1970) demonstrated that the climbing perch, Anabas testudineus showed a metabolic rate of 113.4 ccO₂/kg-hr in water with access to air, 75.5 ccO₂/kg-hr while forcibly submerged, but in water with access to nitrogen, where no additional aerial surface was available for gas exchange, the $\dot{V}O_2$ was 127.5 ccO₂/kg-hr. Thus it appears that surface area per se is not limiting these animals metabolically while in the aquatic phase. The surface area available for aquatic gas exchange certainly must set a limit to the extent of the animal's activity, but this will be considered later.

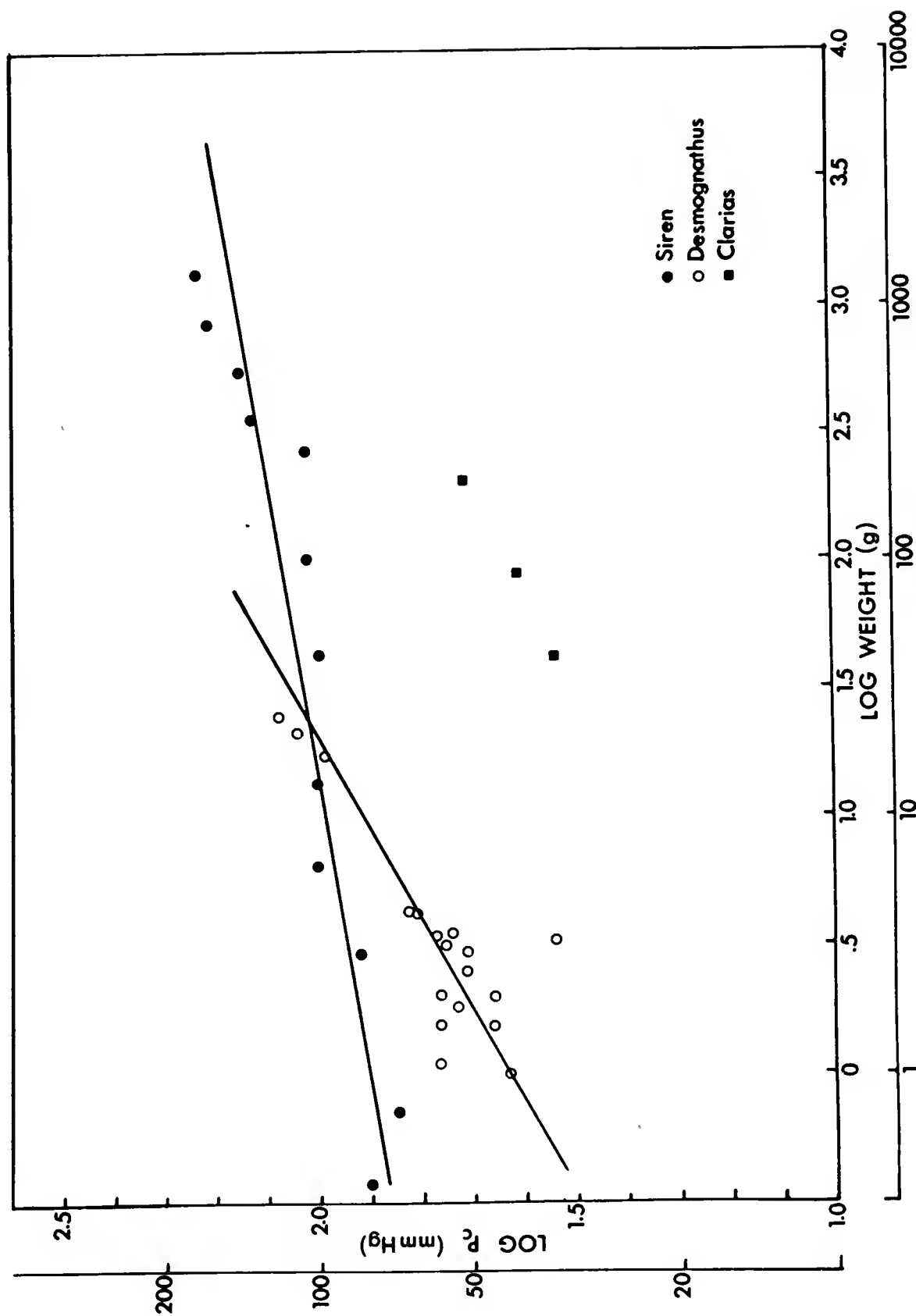
Singh and Hughes (1971) report that the mean $\dot{V}O_2$ of Clarias in a continuous flow of 50% air saturated water drops to almost half the value for fully saturated water. If subjected to hypoxia in a closed chamber, $\dot{V}O_2$ was reduced from 95.6 ccO₂/kg-hr at 151 mmHg to 44 ccO₂/kg-hr at 70 mmHg. These workers further state that Anabas and Clarias resemble I. nebulosus and the toadfish (Opsanus tau) which show oxygen dependent aquatic respiration (oxygen conformance). The data from the present study demonstrate that Clarias regulates its oxygen consumption at a constant rate above water oxygen tensions to about 40 mmHg, suggesting this

species is an oxygen regulator. Similarly, Farber and Rahn (1970) found a linear decrease in water PO_2 , indicating oxygen regulation, even at partial pressures as low as 30 mmHg, in the electric eel, an obligate air breather with reduced gill surface area. Perhaps Singh and Hughes (1971) were observing a decrease in the active metabolism of their animals with decreasing PO_2 of the water. They also stated there was no good correlation between weight and weight-specific oxygen consumption of forcibly submerged fish, which suggests that some of their animals may have been active.

The critical oxygen tension (P_c), or partial pressure of oxygen below which an animal cannot regulate its oxygen consumption, as a function of body weight is plotted in Fig. 13 for Clarias, a transitionally breathing aquatic salamander (Siren), and lungless terrestrial salamanders (Desmognathus). The P_c increases with increasing body weight for salamanders, and appears to follow the same relationship in Clarias. Job (1955) found that the effect of reduced oxygen content on active metabolism was independent of size for Salvelinus fontinalis, while Beamish (1964) makes no mention of any effect of size on the level of P_c in the carp and goldfish, even though he used a weight range of from 17 g-600 g.

Beckenbach (1969) states that the level of P_c depends upon the weight-specific surface area (and therefore, size) of the animal, the metabolic rate, and the temperature. The differences in P_c between terrestrial and aquatic salamanders may be related to the thickness of the skin, as this is the only gas exchange surface in these lungless forms, whereas Siren normally utilizes cutaneous,

Figure 13. Critical oxygen tension of C. batrachus and two salamanders, Siren (from Ultsch, 1972) and Desmognathus (from Beckenbach, 1969), as a function of body weight.



gill and lung respiration. Ultsch (1971) states that forcibly submerged Siren utilize cutaneous respiration almost exclusively. The cutaneous surface to weight relationships for Siren and Desmognathus are virtually identical.

The P_c values of Clarias are about three times lower than those of Siren of a similar weight, even though the metabolic rate of Clarias is approximately 3.7 times higher (for 100 g animals). The differences are probably due to the greatly increased surface area for gas exchange provided by the gills of Clarias.

Singh and Hughes (1971) found that 58.4% of the oxygen uptake is through the air-breathing organ when the fish were in air-saturated water with access to air. However, in the present study, fish that were breathing bimodally in the metabolic chambers did not surface very often, and little oxygen (10-20% of total) was taken in through the air-breathing organs. The event recorder data indicate that the number of surfacings per hour is related to the weight of the fish, water PO_2 , time of day, external weather conditions, and the amount of disturbance to the fish. As oxygen consumption values were taken every hour in the metabolic studies, it is probable that the low values for aerial breathing may be a result of disturbances that inhibited surfacing. All experiments were conducted during daylight hours, and, if they extended into the evening, lighting remained on, a condition which produced low air-breathing values. In one case (71 g individual), the lights were turned off for a few hours in the early evening and the $\dot{V}O_2$ values for aerial breathing doubled. It appears that the gills

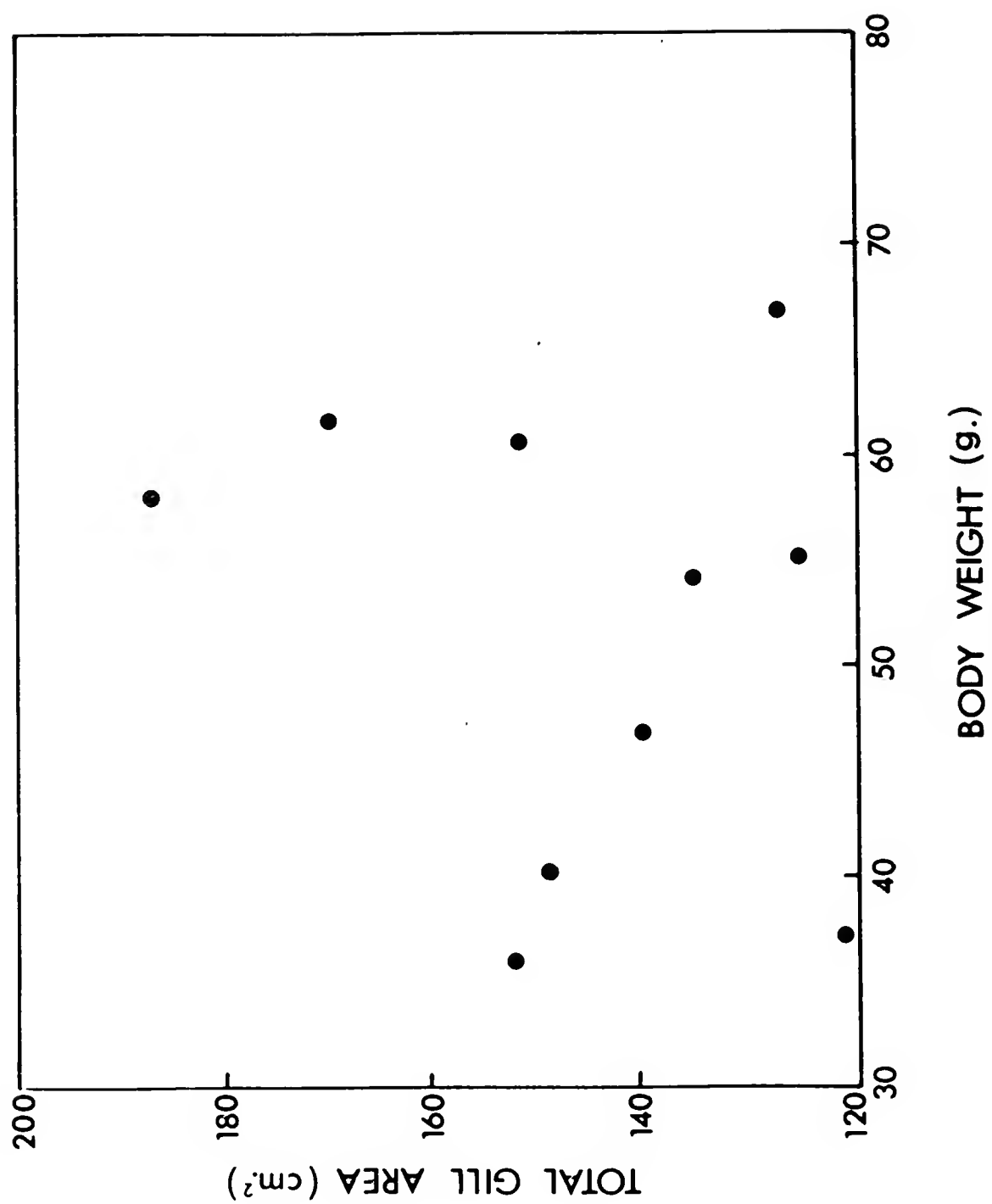
provide adequate respiratory exchange when the animal is not active, but increased oxygen uptake for activity is obtained through the supplemental exchange surface of the air-breathing organ.

Small fish surface more frequently than larger individuals (Fig. 10) apparently requiring proportionally more oxygen from air than larger ones. This is unusual for bimodal breathers. McMahon (1970) found that juvenile lungfish could survive forcible submergence longer than adults and Ultsch (1972) showed that Siren lacertina, an aquatic salamander, can function as a water breather at small sizes only; animals larger than 800 g are obligate air breathers. Although I did not have any large (300 g or larger) animals, which may be obligate air breathers, the size range in this study (30-244 g) indicates that small fish, when given the opportunity, breathe air more often than large ones.

Saxena (1966) measured the gill surface area of three species of Indian catfish; one water breather and two air breathers (including Clarias batrachus). The gill surface area of Rita rita, a water breather, increases curvilinearly with increasing body weight as is the usual relationship, while C. batrachus (Fig. 14) showed no such correlation over a size range of 36-67 g. Perhaps the proportionally higher metabolic rates coupled with gill surface areas that are not proportional to weight in small fish account for the fact that small fish breathe air more often than large fish.

In oxygen deficient water, where the animals are breathing air only, the rates of metabolism are minimal, but can be raised

Figure 14. Gill surface area of C. batrachus as a function of body weight (from Saxena, 1966).



by increasing the frequency of air breathing. In one case, the surfacing frequency was measured by event recorder for a fish placed in nitrogen-saturated water (Fig. 11). The surfacing frequency during the day was approximately equal to the values for surfacing in air-saturated water, but the night values were greatly increased. When the fish are maintained in water with a low oxygen tension, activity is curtailed (during daylight hours) and they swim slowly to the surface for a breath, then fall back to the bottom, often on their sides. Opercular activity is normally diminished or completely stopped.

Oxygen uptake during air exposure is comparable to values for fish in water with access to air. Many air-breathing fishes show reduced $\dot{V}O_2$ in air, while others (such as the African and South American lungfishes and electric eel, which are obligate air breathers, or forms such as Periophthalmus which is active when it leaves water) show no reduction in $\dot{V}O_2$. Clarias is an example of the latter case as they are usually active upon emersion. Clarias is capable of raising the active aerial $\dot{V}O_2$ to at least three times the minimal level. The high levels of oxygen uptake in air are not entirely due to the utilization of the air-breathing organ because Clarias breathing air in oxygen-deficient water show a 27-46% decrease in $\dot{V}O_2$ below values for air-saturated water with access to air. The skin of these fish may assume up to 50% of the oxygen uptake in air. Singh and Hughes (1971) found that 16% of the $\dot{V}O_2$ in air-saturated water was cutaneous. The amount of blood circulating to the skin evidently increases upon emersion, as the skin of albino

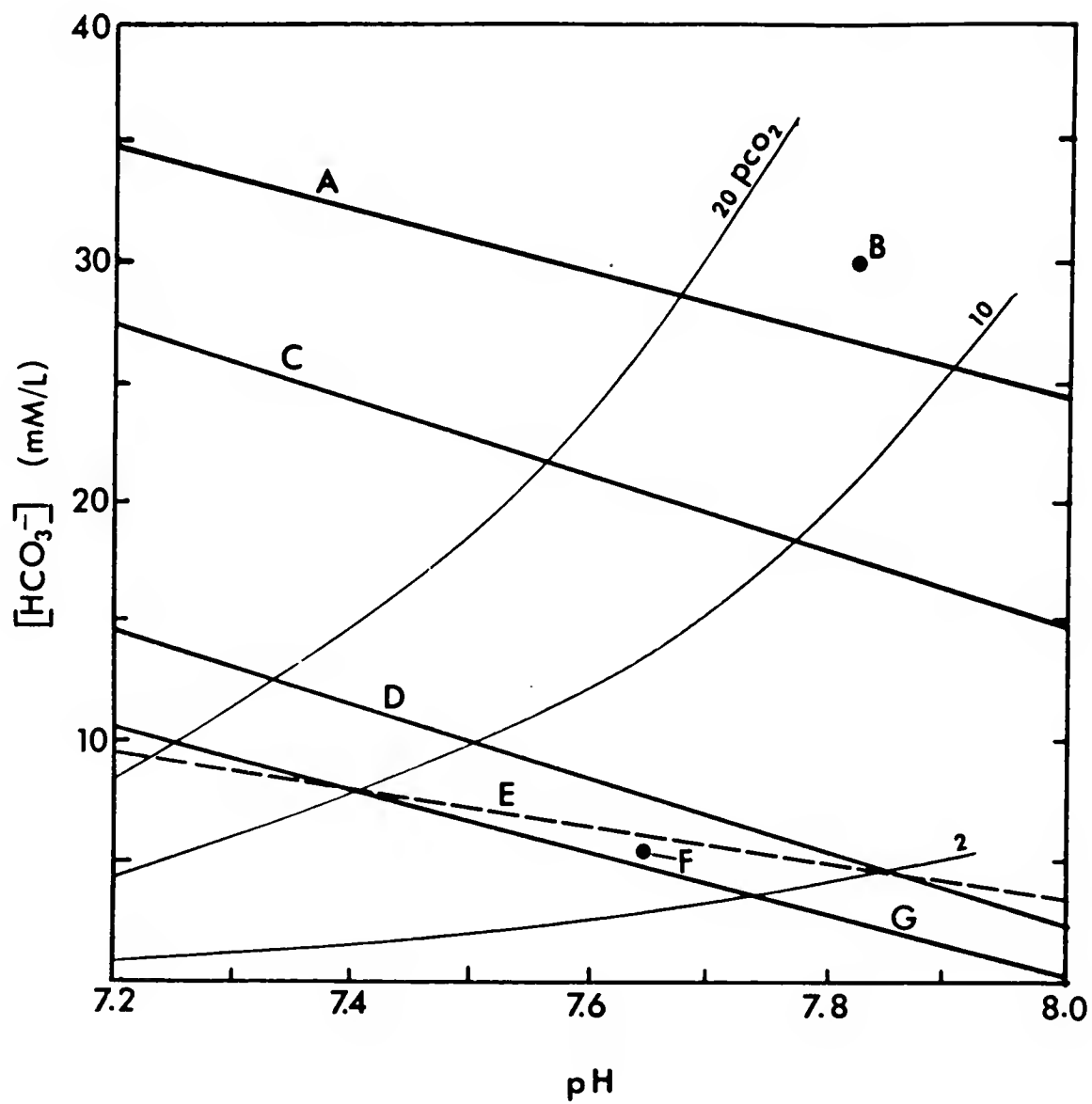
individuals becomes pinker and cutaneous $\dot{V}O_2$ increases. Berg and Steen (1965) found that cutaneous oxygen uptake of Anguilla vulgaris is about 2.7 times greater in air than in water. The gills of Clarias also probably play an important role in aerial respiration, as the widely spaced thickened lamellae would not collapse in air and probably function similarly to those of species that employ gill breathing in air, such as Symbranchus, Hypopomus and Periopthalmus.

The high aerial $\dot{V}O_2$ rates of Clarias batrachus seem somewhat inconsistent with some of the other aspects of the physiology of this fish when it is exposed to air. Singh and Hughes (1971) found that the respiratory quotient of C. batrachus in air was lower (0.52) than other air-breathing forms they examined (Anabas and Saccobranhus) even though Anabas is scaled. Howell (1970) examined the buffering curves of blood of air-breathing fish (including C. batrachus). These data, plus values for the carp and bullfrog (from Rahn, 1967), are shown in Fig. 15; all values are calculated for 25°C. Clarias and Neoceratodus, both primarily water-breathers, have low HCO_3^- concentrations and are not capable of buffering blood carbon dioxide much better than the carp. Howell (1970) states that preliminary laboratory studies indicate that Clarias goes into severe respiratory acidosis when forced to reside in air for a few hours. Yet, Clarias is the only one of these air-breathing fishes that has been reported to leave the water voluntarily.

The skin of Clarias is very sensitive to desiccation and unless it is kept moist, a few hours of air exposure produces necrosis of

Figure 15. Buffering capacity of blood of selected vertebrates at 25°C (from Howell, 1970, and Rahn, 1967).

- A Electrophorus
- B Rana
- C Protopterus
- D Lepidosiren
- E Cyprinus
- F Clarias
- G Neoceratodus



the skin. Both the sensitivity of the skin and a propensity for respiratory acidosis in air is probably why Clarias leaves the water and migrates mainly (if not exclusively) when it is raining. Clarias also follows thin sheets of runoff that flow over roads and down into the ditches. These conditions maintain a film of water over the animal's body surface, which prevents skin damage and presumably would permit carbon dioxide elimination through the naked skin.

In water, Clarias shows coupling of respiratory and circulatory adaptations similar to other air-breathing forms such as Electrophorus, Periophthalmus, and Symbranchus, as maximum cardiac output follows an air breath. Inflation of the gill chamber in Clarias corresponds to an increase in heart rate from about 29 to 39 beats/minute. Electrocardiograms of Clarias show physiological features of a terrestrially active fish such as Periophthalmus. After the experimental tank was drained, heart rate fell to approximately half the initial value until the fish moved and took a breath, then tachycardia ensued with a 250% increase in heart rate. This is very similar to the results obtained by Johansen (1966) for Symbranchus. Gordon et al. (1970) found that the Chilean cling-fish, Sicyases, a fish which is inactive upon emersion, showed an immediate drop in heart rate upon removal from water. Heart rate in this fish remained at low levels for the duration of their emersion.

Clearly, Clarias batrachus has many characteristics that have contributed to its success in South Florida. It is able to survive using aquatic respiration only in aerated water, until the PO_2 drops

to very low levels. Air breathing is more common at night, which tends to decrease susceptibility to predation by diurnally active predators (herons). When oxygen tensions are low, fish survive by breathing air and reducing the amount of activity. They can survive drying (and probably cold in South Florida) by burrowing in mud or making short excursions to deeper water. During the rainy season they make extended excursions by water or land to other waters, which contributes to the dispersal of these species prior to breeding.

SUMMARY

1. Clarias batrachus is a facultative air breather.
2. The $\dot{V}O_2$ of Clarias varies as a function of weight and access to air. The metabolic rates of Clarias in air and water with access to air are essentially the same, but the $\dot{V}O_2$ of forcibly submerged fish is lower.
3. The metabolism characteristic of forcibly submerged Clarias is primarily aerobic, its reduced rate is probably the result of reduced activity.
4. When the fish are in water with access to air, aquatic breathing accounts for 80-90% of the total oxygen uptake. Small fish appear to increase aerial respiration at higher oxygen tensions than do large fish.
5. Clarias are oxygen regulators at oxygen tensions above 40 mmHg.
6. The critical oxygen tension (P_c) of Clarias appears to increase with increasing body weight, as it does in salamanders. Because of the increased surface area for gas exchange provided by the gills of Clarias, the P_c values are about three times lower than those of bimodally-breathing aquatic salamanders (Siren).
7. The number of air breaths per unit time is a function of time of day (doubles at night), and is inversely proportional to the weight of fish. This may be due to the lack of a proportional

relationship between gill surface area and weight that is shown in small Clarias (35-68 gms), and also the proportionally higher metabolic demands of small fish.

8. Oxygen uptake during air exposure is not reduced, as Clarias is active upon emersion. Cutaneous oxygen uptake accounts for up to 50% of the total $\dot{V}O_2$ in air-exposed fish. Cutaneous vasodilation apparently occurs during emersion.
9. The high oxygen capacity of Clarias blood is probably an adaptation to facilitate oxygen uptake because of the thickened and somewhat reduced gill surface area that is necessary for air breathing and terrestrial activity.
10. Electrocardiographic measurements of heart beat frequencies demonstrate that Clarias is similar to other air breathers that are well adapted to emersion.
11. It is suggested that the low HCO_3 levels of the blood of Clarias causing respiratory acidosis after brief periods of air exposure and the sensitivity of the skin to desiccation preclude terrestrial activity in Clarias except on rainy nights when a cutaneous water film (essential for carbon dioxide exchange) can be maintained.

BIBLIOGRAPHY

- Beamish, F. 1964. Respiration of fishes with special emphasis on standard oxygen consumption. III. Influence of oxygen. *Can. J. Zool.* 42: 355-366.
- Beckenbach, A. 1969. "Influence of body size on the respiratory physiology of Plethodontid salamanders." Masters Thesis, Univ. of Fla., Gainesville, Fla.
- Belkin, D. 1961. Anaerobic mechanisms in the diving loggerhead musk turtle, *Sternotherus minor*. Ph.D. Dissertation, Univ. of Fla., Gainesville, Fla.
- Berg, T. and J. Steen. 1965. Physiological mechanisms for aerial respiration in the eel. *Comp. Biochem. Physiol.* 15: 469-484.
- Das, B. 1927. The bionomics of certain air-breathing fishes of India, together with an account of the development of their air-breathing organs. *Phil. Trans. Roy. Soc. London.* 216: 183-219.
- Farber, J. and H. Rahn. 1970. Gas exchange between air and water and the ventilation pattern in the electric eel. *Respir. Physiol.* 9: 151-161.
- Gordon, M., J. Boëtius, D. Evans, R. McCarthy, and L. Oglesby. 1966. Aspects of the physiology of terrestrial life in amphibious fishes. I. The mudskipper, *Periophthalmus sobrinus*. *J. Exp. Biol.* 50: 141-149.
- Gordon, M., S. Fischer and E. Tarifeño. 1970. Aspects of the terrestrial life in amphibious fishes. II. The Chilean clingfish, *Sicyases sanguinensis*. *J. Exp. Biol.* 53: 559-572.
- Hemmingsen, A. 1960. Energy metabolism as related to body size and respiratory surfaces, and its evolution. *Rept. Steno Mem. Hosp. Nord. Insulin Lab.* 9 (Part II).
- Hochachka, P. 1969. Intermediary metabolism in fishes. In Physiology of Fishes. I. ed. W. Hoar, J. Randall. Academic, New York and London.
- Howell, G. 1970. Acid base balance in transition from water breathing to air breathing. *Federation Proc.* 29: 1130-1134.


- Hughes, G. and B. Singh. 1970. Respiration in an air-breathing fish, the climbing perch, Anabas testudineus (Bloch) I. Oxygen uptake and carbon dioxide release into air and water. J. Exp. Biol. 53: 265-280.
- Hughes, G. and B. Singh. 1971. Gas exchange in an air-breathing catfish, Saccobranchus (= Heteropneustes) fossilis. J. Exp. Biol. 55: 667-682.
- Janssens, P. 1964. The metabolism of the aestivating African lungfish. Comp. Biochem. Physiol. 11: 105-117.
- Job, S. 1955. The oxygen consumption of Salvelinus fontinalis. Univ. Toronto Studies Biol. Ser. No. 61. Publ. Ontario Fisheries Research Lab. 73.
- Johansen, K. 1966. Air breathing in the teleost Symbranchus marmoratus. Comp. Biochem. Physiol. 18: 383-395.
- Johansen, K. 1970. Air breathing in fishes. In Physiology of Fishes. IV. ed. W. Hoar, J. Randall. Academic, New York and London.
- Johansen, K., D. Hanson, and C. Lenfant. 1970. Respiration in a primitive air breather, Amia calva. Resp. Physiol. 9: 162-174.
- Johansen, K., C. Lenfant, K. Schmidt-Nielsen and J. Petersen. 1968. Gas exchange and control of breathing in the electric eel, Electrophorus electricus. Z. Vergleich. Physiol. 61: 137-163.
- Junqueira, L., J. Steen, and R. Tinoco. 1967. The respiratory area of the fishes of teleosts from Rio Negro and Rio Branco area. Research Papers from the Alpha Helix Amazon Expedition. B: 20-1.
- Lentfant, C. and K. Johansen. 1968. Respiration in the African lungfish, Protopterus aethiopicus. I. Respiratory properties of blood and normal patterns of breathing and gas exchange. J. Exp. Biol. 49: 437-452.
- Marvin, D. and A. Heath. 1968. Cardiac and respiratory responses to gradual hypoxia in three ecologically distinct species of freshwater fish. Comp. Biochem. Physiol. 27: 349-355.
- McMahon, B. 1970. The relative efficiency of gaseous exchange across the lungs and gills of an African lungfish, Protopterus aethiopicus. J. Exp. Biol. 52: 1-15.
- Mookerjee, H. and S. Mazumdar. 1950. Some aspects of the life history of Clarias batrachus (Linn.). Proc. Zool. Soc. Bengal, Calcutta. 3: 71-79.

- Muir, B. 1969. Gill dimensions as a function of fish size. J. Fish. Res. Bd. Canada 26: 165-170.
- Munshi, J. and B. Singh. 1968. A study of the gill epithelium of certain freshwater teleostean fishes with special reference to the air-breathing fishes. Ind. J. Zool. 9: 91-107.
- Neill, W. 1950. An estivating bowfin. Copeia. 1950: 240.
- Rahn, H. 1967. Gas transport from the external environment to the cell. Ciba Found. Symp. Develop. Lung. pp. 3-29.
- Rose, F. and R. Drotman. 1967. Anaerobiosis in a frog, Rana pipiens. J. Exp. Zool. 166: 427-432.
- Saxena, D. 1960. On the asphyxiation and influence of carbon dioxide on the respiration of air-breathing fish, Heteropneustes fossilis and Clarias batrachus. J. Zool. Soc. India 12: 114-124.
- Saxena, D. 1966. Studies on the physiology of respiration in fishes. VI. Comparative study of the gill area in some freshwater catfishes of India. J. Zool. Soc. India. 16: 38-47.
- Singh, B. and G. Hughes. 1971. Respiration of an air-breathing catfish, Clarias batrachus. J. Exp. Biol. 55: 421-434.
- Smith, H. 1945. The freshwater fishes of Siam or Thailand. Bull. U.S. Natl. Mus., Smithsonian Inst., Washington, D.C., No. 188.
- Sterba, G. 1963. Freshwater Fishes of the World. Viking, New York.
- Ultsch, G. 1972. Gas exchange and metabolism in the Sirenidae (Amphibia, Caudata). Ph.D. Dissertation, Univ. of Fla., Gainesville, Fla.
- Willmer, E. 1934. Some observations on the respiration of certain tropical fresh water fish. J. Exp. Biol. 11: 283-306.

BIOGRAPHICAL SKETCH

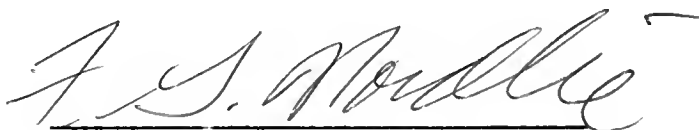
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I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.



B. K. McNab, Chairman
Professor and Chairman of Zoology

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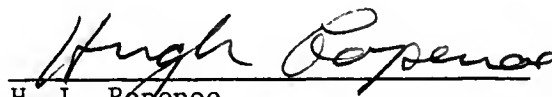
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Professor of Zoology

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This dissertation was submitted to the Department of Zoology in the College of Arts and Sciences and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

June, 1973

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